Influence of Harvesting Time on Yield and Composition of the Essential Oil of a Thyme (*Thymus pulegioides* L.) Growing Wild in Campania (Southern Italy)

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Essential oil of the Italian thyme *Thymus pulegioides* growing wild has been extracted by hydrodistillation from leaves and flowers collected at different growth times. The constituents of the essential oil have been characterized by gas chromatography (GC) and GC−mass spectrometry. Sixty-three compounds have been identified. Essential oils were characterized by a high content of γ-terpinene, *p*-cymene, thymol, and carvacrol which varied from 57.3 to 62.5% of the total oil content. Essential oil yield and composition vary throughout the vegetation time of the plant. The best time to harvest this species of thyme, for both essential oil yield and phenol content, is during or immediately after the full bloom.

Keywords: *Thymus pulegioides; essential oil; harvesting time; γ-terpinene; p-cymene; thymol*

INTRODUCTION

The existence of infraspecific taxa leads to infraspecific chemical differences in the Lamiaceae family (Lawrence, 1978). Among the aromatic plants belonging to the Lamiaceae family, the genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants, some of them bearing vernacular names (Penzig, 1924; Jalas, 1976; Zangheri, 1976; Pignatti, 1982). This taxonomically complex genus of aromatic plants is extensively used, fresh and dried, as a culinary herb. The essential oil serves for the flavoring of all kinds of food products, sauces, meats, and canned foods, for the preparation of some liqueurs, and in perfumery for the scenting of soaps and lotions. Furthermore, it is also used for medicinal purposes, among others, as an antiseptic, an antispasmodic, and an antitussive and for its antimicrobial properties (Van Den Broucke and Lemli, 1981; Janssen *et al.*, 1987; Biondi *et al.*, 1993; Panizzi *et al.*, 1993). The essential oils of some *Thymus* species have been studied by several authors. Granger and Passet (1973) described six chemotypes of *Thymus vulgaris* L. (Sectio *Thymus*) native in France using as the classification criterion the dominant terpene in the oils: geraniol, linalool, α-terpinol, carvacrol, thymol, and the complex *trans*-thujan-4-ol/terpinen-4-ol. Subsequently, they identified a seventh chemotype, growing in the Iberian peninsula, which was characterized by high levels of 1,8-cineole (Adzet *et al.*, 1977). They also confirmed that genetic and ecological factors, especially climatic, determine the distribution frequency of the chemotypes among populations (Granger and Passet, 1973; Adzet *et al.*, 1977; Passet, 1979). Miquel *et al.* (1976) identified in Moroccan thyme oil *Thymus satureioides* borneol as the main constituent of the essential oil and underlined that this particular species of thyme contains a large amount of camphor, giving a noticeable note of camphor to the essential oil. Adzet *et al.* (1991) referred that the essential oil of *Thymus willkomii* Ronninger [Sectio *Serpyllum* (Miller) Bentham.], an endemic Iberian northeastern species, is mainly constituted by linalool, α-terpinenyl acetate, and 1,8-cineole. 1,8-Cineole is also the major component (24.5%) with camphor (22.8%) of *Thymus moroderi* Pau ex Martínez (Sectio *Pseudothymbra* Bentham.), an endemic plant of eastern Spain (Adzet *et al.*, 1989a). *Thymus glandulosus* Lag. ex H. del Villar (Sectio *Thymus*), an endemic plant of southern Spain and northern Africa, is characterized by *p*-cymene as its main component (58.0%) (Adzet *et al.*, 1989b). An investigation of the chemical polymorphism of the essential oils of *Thymus* species from Portugal showed that *Thymus villosus* L. subsp. *villosus* (Sectio *Pseudothymbra*) always has a large percentage of *p*-cymene and also that essential oils of *Thymus capitellatus* Hoffmanns & Link (Sectio *Thymus*) has a composition similar to those of *Thymus camphoratus* Hoffmanns & Link (Sectio *Thymus*), so it is not possible to distinguish these two species by their essential oils (Ribeira Salgueiro, 1992). Leaf essential oil of *Thymus capitellatus* has also been investigated by Figuerido *et al.* (1993) in addition to the oil from *Thymus lotocephalus* G. López & R. Morales. In both oils, 1,8-cineole was the major component. Lately, Salgueiro *et al.* (1995) investigated the composition of the essential oil of eleven populations of *Thymus carnosus* Boiss. (Sectio *Thymus*), a species endemic to southwestern Iberia. Borneol was the main constituent in all the populations, except in one, which had a high content of linalool. Several reports concerning the qualitative and quantitative composition of some *Thymus* species growing in Italy have been published.

In five samples of *Thymus herba-barona* Lois. (Sectio *Serpyllum*) collected from six ecologically different stations, Falchi (1967) identified only two phenols, carvacrol being largely prevalent on thymol, while the oil from Badda Ubbara station was characterized by a very high content of thymol. In two varieties of *Thymus longicaulis* C. Presl (var. *longicaulis* and var. *subisophyllus*) (Sectio *Serpyllum*) collected in different localities of central Italy, Bellomaria *et al.* (1981) identified, by gas chromatography (GC), 24 compounds and showed that between the two varieties some differences exist in the chemical composition of the essential oils. While thymol and carvacrol are present in both varieties, var. *subisophyllus* was more plentiful in α- and β-pinene, camphene, bornyl acetate, and β-caryophyllene. Piccaglia and Marotti (1991) determined by GC and GC−

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mass spectrometry (MS) the composition of the essential oil of *T. vulgaris* L. growing wild in northern Italy and harvested in two consecutive years. They identified 44 components, monoterpenes being the most abundant. The main constituents were *p*-cymene, γ-terpinene, and thymol. This finding was successively confirmed by Panizzi *et al.* (1993). Recently, Biondi *et al.* (1993) reported for *Thymus capitatus* (L.) Hoffmanns & Link (Subgen. *Coridothymus*) a high content (86.33%) of carvacrol. To date, no investigations have been reported on the essential oil of *Thymus pulegioides* L., an endemic Italian species belonging to the Sectio *Serpyllum*. Therefore, in continuation of studies on essential oils from plants growing wild in southern Italy (Senatore and De Feo, 1994a,b; Reverchon and Senatore, 1994, 1995) and as a contribution to a better knowledge of the Italian wild species of the genus *Thymus*, this paper reports on the essential oil from leaves and inflorescences of the *T. pulegioides* L. This plant grows wild in Campania, southern Italy, and in dry meadows, calcareous grassland, and crags of the peninsula Sorrentina and Amalfitan coast (Naples and Salerno provinces) and is commonly used by the local population for various medicinal purposes such as expectorant, anthelmintic, gastric antispasmodic, and astringent (De Feo *et al.*, 1992; De Feo and Senatore, 1993). The decoction of the whole plant is used, externally, as an antirheumatic and against oedemas, while the decoction of the tops is used as a foot bath in sinusitis. Furthermore, its flowers, when soaked in water, on Ascension night, produce a perfumed water wich is used to wash oneself the next day (De Feo *et al.*, 1992). The variation in occurrence of certain compounds in a plant is a function of any one of, or a combination of, three factors: genetically determined properties, the age of the plant, and the environment. Ideally, an experiment evaluating the relative importance of these factors should be designed with randomized blocks and plants of equal age, collected by careful sampling in a random and systematic manner from the natural populations. In several species of Lamiaceae, at flowering, essential oil is at the highest level, while in other species, flowering has a lower influence (Putiewski *et al.*, 1986). The present investigation deals with the variations of yield and composition of the essential oil obtained at different growth times by plants collected from the same stand to limit the influence of the factors previously cited. In order to ascertain the best time to harvest *T. pulegioides* in terms of essential oil yield and concentration of various components, the qualitative and quantitative variations at different periods of the plant growth throughout the months of April−September 1994 have been investigated.

EXPERIMENTAL PROCEDURES

Materials. Thyme leaves and flowers (*T. pulegioides* L.) were collected, in 1994, every second week, in the afternoon, avoiding the days after the rains, as follows: flowers at beginning and during the full flowering and leaves in different times of the growing phase of the plant (from April to mid-September). At each harvest, samples were hand-cut from a same stand of plants growing near Agerola (Lattari mountains, 750 m above sea level, 40° 37′ N and 14° 28′ E). Plant material was collected and authenticated by Dr. V. De Feo (Faculty of Pharmacy, Università di Salerno, Italy), and voucher specimens have been deposited in the Herbarium of the Botany chair, Faculty of Pharmacy, Naples University.

Oil Extraction. The oils were isolated from the fresh, finely chopped, material. Samples were extracted by hydrodistillation, owing to its simplicity and rapidity and relative stability of the analytes (*European Pharmacopoeia*, 1975). Extraction of oil was done the same day as plant sampling.

Analytical Procedures. Volatile thyme oil components were separated for identification by GC and GC−MS.

(a) Gas Chromatography. GC work was performed on a Perkin-Elmer Σ 115 gas chromatograph equipped with an FID and a DB-1 fused-silica column (J&W, Folsom, CA) (30 m × 0.25 mm inside diameter, film thickness of 0.25 μm). GC conditions were as follows: oven temperature, 40 °C for 5 min, subsequently at 2 °C/min up to 290 °C and then held isothermal for 15 min using He as carrier gas. Injector and detector temperatures were 150 and 290 °C, respectively. Samples of 0.1 μL in CH_2Cl_2 were injected.

(b) Gas Chromatography−Mass Spectrometry. GC−MS data were obtained on a Hewlett-Packard 5890 A instrument coupled with a mass selective detector (Hewlett-Packard, Palo Alto, CA, model MSD 5970 HP) operated in electron ionization mode at 70 eV. The HP-1 fused-silica column (25 m × 0.25 mm inside diameter, film thickness of 0.33 μm) was programmed as described above for GC determinations. Mass spectra were also obtained by a Varian Model 3400 gas chromatograph, interfaced with a Finnigan MAT Magnum ion trap detector (ITS 40, Finnigan MAT, San Jose, CA). The column used for this apparatus was a fused-silica DB-5 column (J & W) (30 m × 0.25 mm inside diameter, film thickness of 0.25 μm). The percentage composition of the essential oils was computed from GC peak areas without using correction factors. Qualitative analysis was based on the comparison of the retention times and of the mass spectra with the corresponding data of components of reference oils and pure compounds whenever possible. Mass spectra were compared with those of mass spectra libraries (NBS 43 K, NIST, and WILEY 5).

Preparation of Thymol and Carvacrol Methyl Ethers. Both compounds were prepared by treating in aqueous sodium hydroxide the corresponding phenol (Aldrich) with dimethyl sulfate (Aldrich) as the alkylating agent (*Vogel's Textbook of Practical Organic Chemistry*, 1989). Products were tested by GC−MS as above described.

RESULTS AND DISCUSSION

The fresh leaves of *T. pulegioides* yielded upon hydrodistillation a pale yellow oil which has an agreeable odor, reminiscent of *T. vulgaris* oil. The yields varied between 0.38 and 1.11% on the weight of the fresh leaf material in the season that lasts from mid-April to mid-September. The hydrodistilled fresh flowers produced a yellow oil in a yield of 0.50 and 0.87%, respectively, for the beginning and the full flowering. The harvest times, the yields of oil, and the class of identified components of hydrodistilled oil samples have been listed in Table 1. The repeated samplings in the present investigation show that there is a certain temporal variation in the occurrence of some compounds but also that most of the compounds detected were always present. The results of the GC−MS analyses are given in Table 2, compounds being listed in order of their elution time on the HP-1 column. A total of 63 components has been identified, and these compounds accounted for 94.1−97.4% (leaves) and 96.8−97.2% (flowers, respectively, at the full and at the beginning of the flowering) of the peak area of the chromatographic profiles in the examined samples. Generally in the area of sample collection, flowering of *T. pulegioides* starts at the end of April; beginning in May and for a period of almost 4 weeks (from mid-May to mid-June), plants are in full bloom. In this time, in the fourth week of May, the essential oil content in the leaves increases to its highest level (1.11%), then decreases, but increases again to another high level (0.93%) in the third week of June. From July to September, the leaf essential oil contents slowly decrease. This finding thus confirms

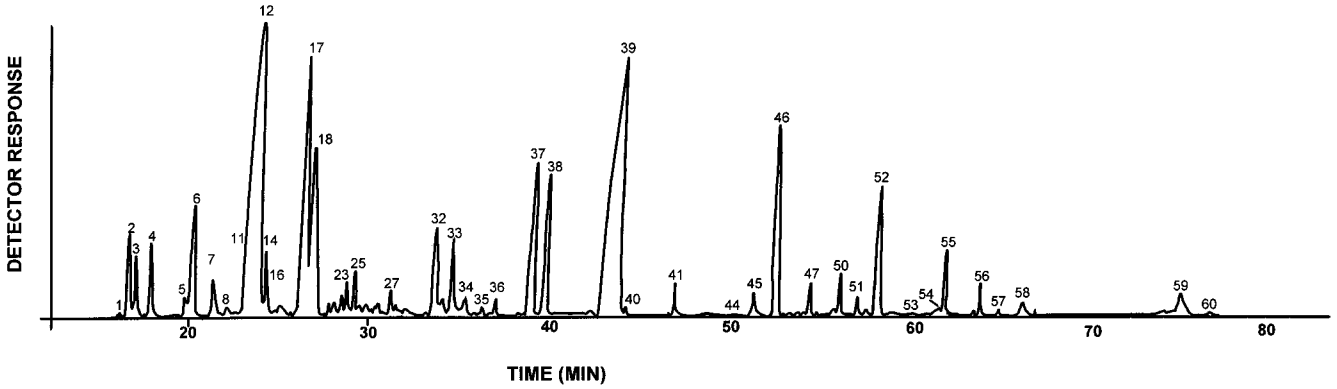

Figure 1. Gas chromatogram of the leaf thyme oil of 2/5. See Table 2 for identifications.

Table 1. Yields[a] and Percentage Composition[b] of Essential Oils from *T. pulegioides* L. Samples Growing Wild

	date of harvest													
	Apr 18[c]	May 2[c]	May 12[c]	May 12[d]	May 24[c]	May 31[c]	May 31[d]	June 10[c]	June 21[c]	July 7[c]	July 20[c]	Aug 2[e]	Aug 19[c]	Sept 13[c]
yield of oil	0.38	0.71	0.84	0.50	1.11	1.01	0.87	0.90	0.93	0.87	0.78	0.70	0.68	0.59
MH	50.3	41.3	39.6	37.4	32.6	29.2	29.9	33.6	36.8	39.3	37.8	36.3	42.2	44.8
OCM	32.4	39.3	43.0	46.8	45.0	45.9	52.4	43.2	40.9	39.4	40.3	39.4	35.6	34.9
p-cymene + γ-terpinene	43.3	33.7	32.3	30.4	28.1	23.1	25.1	27.9	30.0	32.5	33.2	33.5	37.5	38.7
thymol + carvacrol	19.2	23.6	26.5	33.9	32.5	36.2	43.3	33.9	31.6	29.3	27.4	25.2	24.0	22.4
TPC	26.8	31.7	34.3	43.4	39.4	40.8	49.4	38.4	35.7	33.4	31.2	29.7	27.9	26.8
SH	7.6	9.9	8.2	7.5	8.8	8.4	8.8	7.9	8.6	8.1	8.3	8.0	8.8	7.4
OCS	0.6	1.6	1.6	0.8	2.3	1.1	0.6	1.3	1.5	1.9	0.9	0.7	1.3	0.7
others	4.2	4.5	3.7	4.9	6.8	12.9	5.2	9.3	6.4	7.9	9.6	11.1	10.1	10.0
undetermined	5.0	3.5	4.1	2.7	4.9	2.8	3.3	4.9	6.2	3.6	3.5	4.8	2.6	3.0

[a] g/(100 g of fresh material). [b] GC peak areas percentage. [c] Leaf. [d] Flowers. MH, monoterpene hydrocarbons; OCM, oxygen-containing monoterpenes; TPC, total phenol content; SH, sesquiterpene hydrocarbons; OCS, oxygen-containing sesquiterpenes.

that the recovery of oil depends on the time of harvest and that the best time to harvest *T. pulegioides* is during the full flowering.

The analysis showed that the oils are rich in monoterpene hydrocarbons and phenolic monoterpenes. Before the early flowering stage, the monoterpene hydrocarbon content peaked at 50.3%. Successively, at the beginning and during the flowering, this value decreases, but it increases again when the flowering was reduced. In all samples, the most abundant monoterpenes were γ-terpinene and *p*-cymene, the biogenetic precursors (via enzymic hydroxylation) of the phenolic terpenes thymol and carvacrol (Poulose and Croteau, 1978a,b). The content of oxygenated monoterpenes, excluding phenolic monoterpenes, shows a maximum (8.7%) in mid-May, at the onset of the full flowering; it decreases to the minimum (4.9%) during the flowering and then increases, in September, when the flowering had finished, it is 7.2%. Borneol, linalool, and 4-terpinol were the major components of this group of compounds. Borneol was present in all samples, in appreciable amounts, while its derivative camphor was detected only in some samples in low amounts or trace. Among the monoterpene alcohols, it is worth mentioning the presence of *cis*- and *trans*-*p*-menth-2-en-1-ol; the latter was already described in trace amounts in flowers and leaves of *Thymus capitellatus* (Figuerido *et al*., 1993), while the former is reported for the first time in the literature on *Thymus* spp. 1,8-Cineole was the unique monoterpene oxide detected, and it was present, in low amounts or trace, only in the oils obtained from the leaves picked after the full bloom. All the essential oils isolated contain two compounds whose concentrations vary between 1.2 and 5.1% for the first and 2.2 and 4.3% for the second. Their GC retention times are between those of dodecene and thymol. The mass spectra of these compounds showed fragmentation patterns which were similar to those reported in literature (Adams, 1989) for thymol methyl ether and carvacrol methyl ether, respectively. Furthermore, the retention time and the mass spectrum of the first of these compounds are superimposable to that previously obtained for a component of the essential oil of *Chritmum maritimum* L. identified as thymol methyl ether (Senatore and De Feo, 1994b). Both of them were already described in other *Thymus* spp., but only in trace amounts (Adzet *et al*., 1989b) or from 1.4 to 2.5% (Piccaglia and Marotti, 1991; Mc Gimpsey *et al*., 1994) the thymol methyl ether and in trace amounts (Adzet *et al*., 1989a) or 1.4% (Miquel *et al*., 1976) the carvacrol methyl ether. Both compounds have been synthesized as described in Experimental Procedures. A comparison of the retention times and mass spectra of thusly obtained compounds confirmed the structures of thymol methyl ether [t_R 39.2 min; MS *m*/*z* (%) 149 (100), 164 (24), 91 (21), 119 (13), 117 (9), 77 (9), 134 (8)] and carvacrol methyl ether [t_R 40.1 min; MS *m*/*z* (%) 149 (100), 164 (33), 91 (23), 117 (13), 77 (11), 119 (10), 134 (10)]. The essential oil from flowering tops shows a higher content of monoterpene hydrocarbons than the one of leaf oil, and at full bloom, this value is the highest (52.4%) in all the examined oils. During full bloom, the total phenol content in flowering tops peaks at 49.6%, but also the level of these compounds at the beginning of the flowering is higher than the ones showed by the leaf oils. Sesquiterpene compounds are recognized as substances which influence insect behavior, and β-caryophyllene has been reported to exhibit attractant properties for the boll weevil (Buttery and Ling, 1984). In this study, the leaf sesquiterpene content varied from 8.1 to 11.5%, while in the flowers, oil is 8.3 and 9.4%, respectively, at the beginning and in the full flowering.

Hydrocarbons are the main constituents of this fraction, with β-caryophyllene and β-bisabolene as the most

Table 2. Percentage Composition of Essential Oils from the Leaf and Flower of *T. pulegioides* L.[d]

	date of harvest													
	Apr 18[a]	May 2[a]	May 12[a]	May 12[b]	May 24[a]	May 31[a]	May 31[b]	June 10[a]	June 21[a]	July 5[a]	July 20[a]	Aug 2[a]	Aug 19[a]	Sept 13[a]
1 tricyclene	0.1	0.1	0.1	tr	0.2	0.1	tr	tr	0.1	0.2	tr		0.2	0.3
2 α-thujene	1.5	1.3	1.7	2.4	1.0	1.0	1.1	1.4	1.5	0.8	0.4	0.4	0.7	0.6
3 α-pinene[c]	1.3	1.5	1.1	0.5	0.3	0.3	0.4	0.5	0.6	0.6	0.7	0.2	0.5	0.2
4 camphene[c]	1.4	1.5	1.4		0.2	0.1	0.4	0.1	0.3	0.4	0.5	0.2	0.2	0.1
5 sabinene[c]	0.2	0.3	0.3	tr	0.1	0.1	0.2	tr	0.2	0.2	0.3	0.1		0.1
6 1-octen-3-ol[c]	4.0	3.0	2.5	3.3	2.4	1.6	2.0	3.2	3.0	3.0	2.5	3.7	3.3	2.4
7 β-myrcene[c]	1.2	0.9	0.7	3.5	0.8	0.7	1.1	0.8	1.0	1.1	1.0	0.5	0.4	0.4
8 δ^2-carene	0.1	0.2	0.3		0.1	tr			0.2	0.2	0.3	0.2	tr	0.2
9 α-phellandrene[c]			0.1	tr	0.1	0.1	0.3		0.1	0.1	tr	0.2		0.3
10 δ^3-carene[c]			0.1		0.2	0.1		tr	0.1	0.1		0.3		0.1
11 α-terpinene[c]	tr	0.1	0.1	0.1	0.2	2.2	0.1	1.9	1.4	1.7	0.1		0.2	0.5
12 *p*-cymene[c]	26.7	21.0	22.1	11.6	18.4	9.6	11.3	13.0	15.4	17.0	19.8	18.6	20.2	20.6
13 cineole-1,8										0.3	0.7	0.5	0.2	0.7
14 β-phellandrene	0.6	0.5	0.5		0.3	0.4	0.3	0.3	0.4	0.4	0.3		0.1	0.7
15 undecene											0.3	0.1	tr	0.2
16 limonene[c]	0.6	0.7	0.7		0.4	0.5	0.4	0.5	0.5	0.5	0.3	0.5	0.2	0.3
17 γ-terpinene[c]	16.6	12.7	10.2	18.8	9.7	13.5	13.8	14.9	14.6	15.5	13.4	14.9	18.9	19.8
18 *cis*-menth-2-en-1-ol		0.2	0.6	0.9	1.0	1.1	0.8	1.2	1.4	1.3	2.1	1.8	1.3	1.1
19 dimethyl styrene		0.2	0.2		0.3		0.2		0.1	0.2	0.1			
20 a methyl undecane											0.5	0.2		
21 terpinolene[c]		0.3	tr	0.5	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.4	0.3
22 *p*-cymenene									tr		0.4	tr	0.2	0.3
23 terpinen-4-yl acetate		0.5	0.7		0.3	0.4	0.4	0.3	0.2		tr	0.6	0.2	0.4
24 *p*-menth-2-en-1-ol			tr	0.6	0.1	0.3	0.5	0.4	0.5	0.5	0.4	0.5	0.3	0.6
25 linalool[c]	0.9	1.0	1.2	0.8	0.6	0.7	0.3	1.0	1.2	1.0	1.4	1.5	0.7	0.5
26 a dodecane			0.2			1.0	0.1	0.7		0.7				0.3
27 camphor[c]	0.2	0.3	0.5		0.4	0.6	0.7	0.4			tr	tr		tr
28 2,4-dimethyldecane											0.4			
29 3,4-dimethyldecane											0.3	0.4		0.5
30 3,6-dimethyldecane											0.2			
31 pinocarveol	0.2	0.2	0.3		0.1	0.2		0.1						
32 borneol[c]	3.9	3.3	3.1	0.8	1.1	0.5	0.1	0.8	1.3	2.2	2.8	3.0	3.6	3.3
33 terpinen-4-ol		1.5	1.6	0.3	1.0	0.5	0.2	0.3	0.5	0.6	0.5	1.5	0.9	1.1
34 *p*-cymen-8-ol	0.4	0.6	0.7		1.0	0.8		0.4	0.1	0.1	tr	0.3	0.3	0.2
35 dodecene[c]		0.2	0.2	0.5	0.4	0.7	0.3	0.5	0.4	0.3	1.2	1.0	1.5	2.1
36 dodecane[c]										tr	0.3	0.2	0.3	0.4
37 thymol methyl ether	4.2	4.2	3.9	5.1	3.3	2.1	2.4	2.0	1.4	1.2	1.4	1.4	1.3	1.6
38 carvacrol methyl ether	3.0	3.5	3.6	4.3	3.1	2.3	3.5	2.2	2.5	2.8	2.9	2.7	2.4	2.8
39 thymol[c]	18.7	23.2	25.5	31.0	31.2	33.8	39.1	31.7	29.9	27.5	25.2	23.1	21.8	20.8
40 carvacrol[c]	0.5	0.4	1.0	2.9	1.3	2.4	4.2	2.2	1.7	1.8	2.2	2.1	2.2	1.6
41 thymol acetate	0.4	0.4	0.3	0.1	0.5	0.2	0.2	0.3	0.2	0.1	0.2	0.1	0.1	tr
42 cuminaldehyde											0.2	tr	0.2	0.2
43 thymoquinone											0.3	0.3	0.1	tr
44 bourbonene		0.1	tr		0.1	0.1		tr		0.1	tr	tr	0.3	0.2
45 tetradecene[c]	tr	0.3		0.5	0.5	1.1	0.5		0.6	0.5	0.5	1.8	1.4	1.0
46 β-caryophyllene[c]	5.2	5.4	5.4	2.7	4.8	4.4	4.9	4.7	4.3	5.0	5.3	5.2	5.4	4.9
47 α-humulene	tr	0.5	0.4	0.2	0.4	0.5	0.7	0.4	0.5	0.6	0.8	1.0	0.8	0.4
48 *allo*-aromadendrene		0.1	tr		0.2	0.1	tr	0.1	tr	tr	0.1	tr	0.1	0.2
49 γ-gurjunene				tr	0.1	tr	0.1	tr	0.2	0.1	tr		0.2	tr
50 γ-muurolene		0.5	0.4	0.6	0.5	0.3	1.0	0.4	0.4	0.2	0.4	0.2	0.2	0.3
51 γ-elemene		0.3	tr	0.2	0.7	0.9	0.4	0.8	1.0	0.7	0.2	0.1	0.3	tr
52 β-bisabolene	2.4	2.9	2.0	3.8	1.8	1.8	1.6	1.5	1.8	1.2	1.4	1.5	1.5	1.2
53 δ-cadinene		0.1	tr	tr	0.2	0.3	0.1	tr	0.4	0.2	0.1	tr	tr	0.2
54 spathulenol	tr	0.2	0.4	0.4	0.6	0.3	0.2	0.4	0.6	0.8	0.5	0.6	0.8	0.4
55 caryophyllene oxide[c]	0.4	1.3	1.0	0.4	1.5	0.7	0.4	0.9	0.8	1.1	0.3	tr	0.4	0.3
56 hexadecene[c]	0.2	0.5	0.8	0.5	1.1	2.5	0.8	2.0	1.0	1.0	0.8	1.3	1.0	1.6
57 *t-t*-farnesol	tr	0.1		tr	0.1	tr			0.1	tr	0.1	0.1	0.1	
58 β-bisabolol	0.2	tr	0.2	tr	0.1	0.1								
59 octadecene[c]		0.3		0.1	0.9	2.6		1.4	0.8	1.2	1.0	0.9	1.0	0.8
60 octadecane[c]		tr		tr		tr	0.7	0.6	tr	0.1	0.5	0.4	0.4	0.3
61 palmitic acid[c]		tr			0.7	1.0	0.3	tr	0.2	0.4	0.1	0.3	tr	
62 eicosene[c]		0.2			0.6	1.6	0.4	1.0	0.4	0.5	0.5	0.5	0.5	0.4
63 docosene[c]		tr			0.2	0.8	0.1	0.9	tr	0.2	0.5	0.3	0.7	0.5

[a] Leaf. [b] Flowers. [c] The compound was identified by comparison of it with the reference substance on the basis of retention time and mass spectrum. [d] GC peak area percentage; tr, trace; peak area % smaller than 0.05%.

abundant components. Between oxygenated sesquiterpenes, caryophyllene oxide is the most abundant component while smaller amounts of spathulenol, *t-t*-farnesol, and β-bisabolol have been detected. The presence of the last two compounds, besides the presence of thymol acetate, represents the first reported occurrence of those compounds in the literature on *Thymus* spp. The total phenol content of *T. pulegioides*, mainly consisting of by thymol, carvacrol, and their corresponding methyl ethers, ranges from 26.9 to 41.1% in the leaf oils and from 43.5 to 49.6% in that from flowers. Thymol is always the main component of this fraction. As expected, the variation pattern of these compounds is inversely related to the γ-terpinene and

p-cymene pattern; i.e., a rise of the phenolic contents accompanied the decrease in the γ-terpinene and *p*-cymene content. The phenol content starts to increase at the beginning of the flowering, attains its maximum in the last week of May, and shows its greatest values during the full flowering period of the plant. In the same period, the value of *p*-cymene + γ-terpinene decreases to the minimum (23.1%) which is attained in concomitance with the highest phenol content. After the full flowering, the phenol content decreases while the *p*-cymene + γ-terpinene content increases. It is also noteworthy that the highest phenol contents coincide with the highest oil yields. This finding confirms that the best time to harvest *T. pulegioides*, for both the oil content and its pharmacological action, which in thyme oils is connected with the phenol content (Van Den Broucke and Lemli, 1981), is during or immediately after the full bloom. Up to now, Italian Pharmacopoeia (1991) requires a minimum percentage (30%, v/v) of phenols in the volatile oil (*Thymi aetheroleum*) obtained from *T. vulgaris* L. and/or *Thymus zygis* L. Therefore, *T. pulegioides* essential oil obtained during the full flowering or immediately before and after this time could be considered a good source of thyme oil for pharmaceutical purposes, also for its content in linalool and limonene; both compounds have an antimicrobial action that may synergize the antimicrobial action of thymol and carvacrol. In conclusion, it can be stated that components of the essential oil of *T. pulegioides* show fluctuations in their relative amounts throughout the period under study; the most conspicuous ones are recorded for monoterpene hydrocarbons *p*-cymene and γ-terpinene and for the phenols thymol and carvacrol. These compounds are found to be alternatively the main components of the oil: monoterpene hydrocarbons before and after the flowering and phenols during the flowering. However, their total content is nearly constant, ranging fom 57.3 to 62.5%. It is also remarkable that some minor components, 1,8-cineole, cuminaldehyde, and thymoquinone, are present in the samples gathered during August and September, whereas β-bisabolol seems to be characteristic for samples gathered before or during the beginning of the flowering.

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